Identification of TUB as a novel candidate gene influencing body weight in humans

Brief Genetics Report

Identification of *TUB* as a Novel Candidate Gene Influencing Body Weight in Humans

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Previously, we identified a locus on 11p influencing obesity in families with type 2 diabetes. Based on mouse studies, we selected *TUB* as a functional candidate gene and performed association studies to determine whether this controls obesity. We analyzed the genotypes of 13 single nucleotide polymorphisms (SNPs) around *TUB* in 492 unrelated type 2 diabetic patients with known BMI values. One SNP (rs1528133) was found to have a significant effect on BMI (1.54 kg/m^2, $P = 0.006$). This association was confirmed in a population enriched for type 2 diabetes, using 750 individuals who were not selected for type 2 diabetes. Two SNPs in linkage disequilibrium with rs1528133 and mapping to the 3′ end of *TUB*, rs2272382, and rs2272383 also affected BMI by 1.3 kg/m2 ($P = 0.016$ and $P = 0.010$, respectively). Combined analysis confirmed this association ($P = 0.005$ and $P = 0.002$, respectively). Moreover, comparing 349 obese subjects (BMI >30 kg/m^2) from the combined cohort with 289 normal subjects (BMI <25 kg/m^2) revealed that the protective alleles have a lower frequency in obese subjects (odds ratio 1.32 [95% CI 1.04–1.67], $P = 0.022$). Altogether, data from the tubby mouse as well as these data suggest that *TUB* could be an important factor in controlling the central regulation of body weight in humans. *Diabetes* 55:385–389, 2006

Until recently, type 2 diabetes was considered a disease of the elderly. Currently, worldwide, a large proportion of newly diagnosed patients are adolescents. The increase in the prevalence of type 2 diabetes, as well as the rapid spreading to a younger age at onset, is largely due to environmental factors, including modern eating habits and reduced physical activity. Obesity is a major risk factor for type 2 diabetes, and the obesity epidemic coincides with the type 2 diabetes epidemic. Genetic studies have suggested a common basis for both diseases. Recently, we identified a locus on 11p15 (95% CI 4,868,745–10,676,565 bps) influencing obesity in families with type 2 diabetes (1). The *TUB* gene was found to be the most relevant candidate gene within this locus, and we hypothesized that *TUB* was associated with obesity susceptibility.

TUB is the founding-member of the tubby-like proteins and is conserved among vertebrate genomes (2). Interestingly, a loss of function mutation of *tub* results in the tubby mouse syndrome, which is characterized by late-onset obesity and neurosensory deficits. The tubby mouse begins to diverge in weight at ~12 weeks of age and ultimately reaches twice the weight of its wild-type littermates (3). Along with weight gain, tubby mouse shows insulin resistance, although it is not overtly diabetic. The high level of *Tub* expression in the hypothalamus, a brain region that is implicated in the control of systemic energy regulation (4), indicates that the obesity phenotype might result from defects in neuroendocrine control of satiety or metabolism. Recent experiments in the worm *C. elegans* show that *tub-1* can increase adiposity when ablated (5). *TUB* is an attractive candidate gene for obesity as it is an important and fundamental factor in metabolism and obesity, although the role of *TUB* in obesity in humans has not been established.

To investigate the role of *TUB* in human obesity, 13 single nucleotide polymorphisms (SNPs) around *TUB* (Fig. 1*A* and online appendix Table 1 [available from http://diabetes.diabetesjournals.org]) were genotyped in 492 unrelated type 2 diabetic patients from the Breda cohort, for whom BMI values were available (Table 1). Linear regression analysis, adjusted for age and sex, revealed that the minor allele of SNP rs1528133 had a marked effect on BMI (+1.54 kg/m^2, $P = 0.006$) (Fig. 1*B*, Table 2). SNP rs1528133 is located 22 kb distal to *TUB* in the flanking gene *RIC3*, which has an unknown function. SNP rs1528133 is in strong linkage disequilibrium with SNPs in the 3′ end of *TUB* (Fig. 1*C*), indicating that the COOH-terminus of *TUB* may be associated with obesity. A replication study of rs1528133 and four SNPs located in the 3′ of *TUB* and in linkage disequilibrium with rs1528133 (rs2272382, rs2272383, rs3750955, and rs1406095) was performed in a population enriched for type 2 diabetes Dutch cohort (RIVM) of 750 individuals (Table 1). We were able

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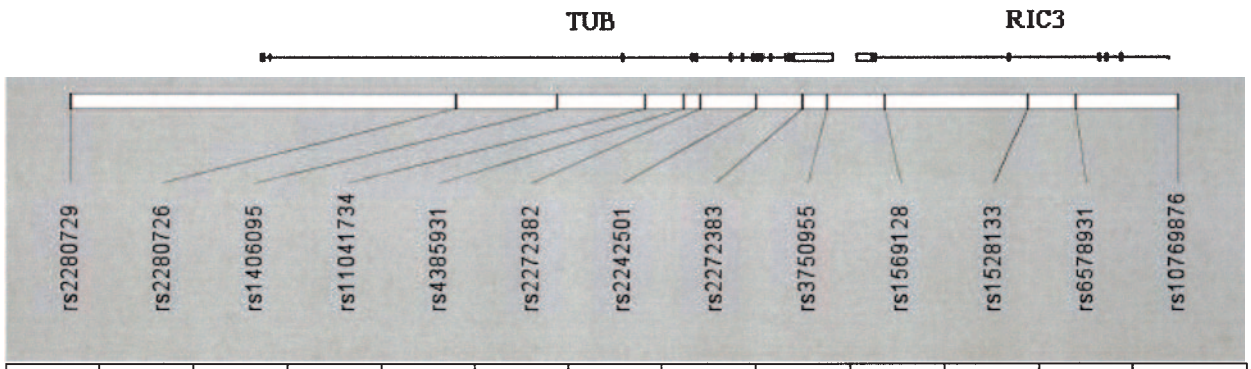
Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

SNP, single nucleotide polymorphism.

A



B

Location	7993782	8039305	8051258	8061649	8066224	8068116	8074679	8080075	8082818	8089461	8106329	8111940	8123921
Breda	0.00 0.30	-0.03 0.88	0.16 0.62	0.04 -0.03	0.06 -0.42	0.08 -0.97	0.28 0.17	0.41 -0.69	0.07 -0.38	0.33 -0.56	**1.54 *** -0.32	0.23 -0.28	0.08 0.05
RIVM			-0.03 -0.36			0.41 **-1.28 ***		0.01 **-1.26 ***	-0.21 -1.32		-0.12 -0.01		
Combined			0.02 0.06			0.35 **-1.12 ***		0.21 **-1.11 ***	-0.07 -0.93		0.54 0.12		

C

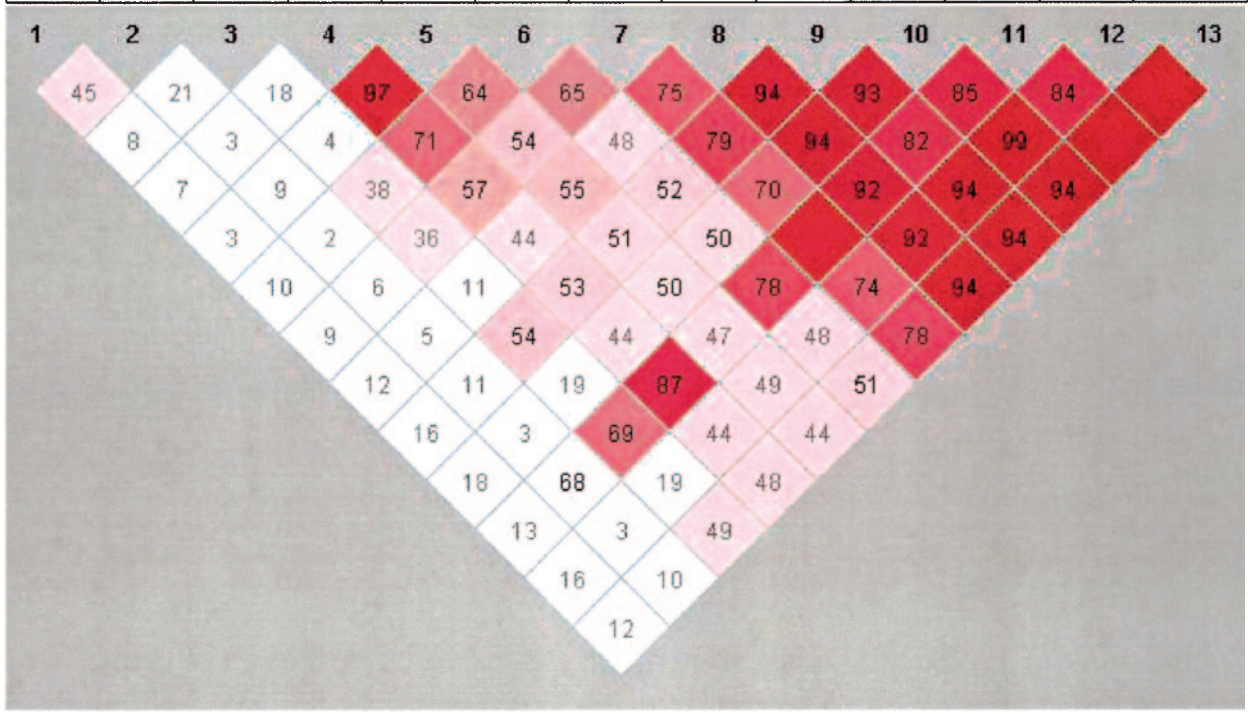


FIG. 1. Fine-mapping study with SNPs in and surrounding the *TUB* gene. *A*: A 130-kb region (7,993,782–8,123,921 bp) encompassing the *TUB* gene and the location of the 13 SNPs used in the fine mapping study. The spacing between the SNPs reflects the distances between them. *B*: The effect of each of the SNPs on BMI (kg/m^2) for the minor allele is indicated in the *upper panel* and the effect on BMI (kg/m^2) for the major allele is indicated in the *lower panel*, for the two cohorts (Breda and RIVM) as well as the combined cohort. *Significant *P* values. *P* values were calculated using the two-sided χ^2 test. *C*: Pairwise linkage disequilibrium between the 13 SNPs, given by the D′ statistics computed with the genotype data from the control cohort of 356 subjects.

to validate our initial result and found a significant effect on BMI for the major alleles of both rs2272382 (−1.28 kg/m^2, $P = 0.016$) and rs2272383 (−1.26 kg/m^2, $P = 0.01$) (Fig. 1*B*, Table 2). These two SNPs are in a strong linkage disequilibrium with rs1528133 (D′ = 0.8 and D′ = 0.9, respectively). Combining both cohorts (n = 1,242) strengthened the effect, as rs2272382 and rs2272383 showed even stronger association with BMI (−1.12 kg/m^2, $P = 0.005$, −1.11 kg/m^2, $P = 0.002$) (Fig. 1*B*, Table 2). These two SNPs remained significant in the combined cohort after stringent Bonferroni correction for five SNPs ($P_c < 0.05/5 = 0.01$).

Comparing the 349 obese subjects from the combined cohort (BMI >30 kg/m^2) with 289 normal subjects (BMI <25 kg/m^2) revealed that the minor allele of SNP rs2272382 was significantly more common in the obese subjects (37.3%) than normal subjects (31.1%) (odds ratio 1.32 [95% CI 1.04–1.67], $P = 0.022$), while the minor allele of rs2272383 showed borderline significance (1.23 [0.98–1.55], $P = 0.078$) (Table 3). In addition, the genotype

TABLE 1
Clinical characteristics of study subjects

Trait	Breda cohort		RIVM cohort	
	Nondiabetic subjects	Type 2 diabetic subjects	Nondiabetic subjects	Type 2 diabetic subjects
n (male/female)	356 (173/183)	492 (224/267)*	341 (197/144)	409 (259/150)
Age at study (years)	49.39 ± 11.68*	70.3 ± 9.9	54.78 ± 6.63	57.1 ± 6.1
Age at diagnosis (years)	—	63.0 ± 11.3	—	53.2 ± 6.6†
BMI (kg/m^2)	NA	27.8 ± 4.1	26.5 ± 3.6	29.6 ± 4.5
n (BMI >30 kg/m^2)	NA	129	48	172
n (BMI <25 kg/m^2)	NA	125	111	53

Data are means ± SD, unless otherwise indicated. *Not available for one subject. †Only available for 189 subjects. NA, not available.

homozygous for the minor allele of SNP rs2272382 was also significantly more common in the obese subjects (14.2%) compared with normal subjects (7.1%) ($P = 0.016$) (online appendix Table 2). Notably, the RIVM cohort includes ~55% type 2 diabetic subjects. In a case-control study comparing patients with type 2 diabetes with healthy subjects from the Breda cohort for all 13 SNPs and RIVM cohort for all five SNPs, we found no associations (data not shown), clearly confirming the gene's direct influence on body weight independent of type 2 diabetes. Therefore, the data suggests that *TUB* may influence BMI in general and may not be involved in type 2 diabetes directly.

The two associated SNPs, rs2272382 and rs2272383, are located in the highly conserved COOH-terminal domain of tubby, which contains the DNA-binding domain and the phosphatidylinositol-binding region. Strikingly, mutations in the murine *TUB* allele and tubby-like proteins were found to be in the same region (6,7). Comparing the linkage disequilibrium plot (D′) between these three SNPs in the Breda case subjects and the case and control subjects from RIVM revealed no major differences (data not shown). Moreover, the allele frequencies were similar. It is therefore possible that the differences between the Breda and RIVM cohorts can be attributed to different patterns of association between the marker allele and the true, not yet identified, disease-associated allele. Alternatively, it is possible that different alleles at the same locus are responsible for increased risk in different populations.

It is tempting to speculate that a variation in this region may have affected a domain important to the biological function of tubby. However, it should be noted that the region showing association also encompasses the *RIC3* gene, which is known to be involved in neurotransmission (8). Although we cannot formally exclude that *RIC3* is contributing to body weight, the *TUB* gene is a more attractive candidate gene for body weight due to its function.

To our knowledge, the potential contribution of *TUB* to human obesity was assessed only once (9). The authors have examined five polymorphic microsatellite markers around *TUB* in 716 Pima Indians comprising 217 nuclear families for sibpair linkage to BMI. No significant linkages were found in an analysis of all sibships or in an analysis restricted to discordant sibpairs. However, this linkage study was done in a different population and not with the same SNPs that were used by us.

Thus, the role of tubby as an important factor in metabolism and obesity has not been shown in humans. Our study is the first to provide an initial estimate of the association of the *TUB* gene with a quantitative measure of obesity. Recently, the effect of *MC4R*, a major gene for obesity, on weight regulation was estimated to be −0.52 kg/m^2 (10), and since the effect of *TUB* on weight regulation is more then twice as high (−1.3 kg/m^2), this indicates the importance of *TUB*'s contribution to polygenetically regulated body weight.

Altogether, data from the tubby mouse as well as these data suggest that tubby has a protective role in the central regulation of body weight in humans. Mutations in *TUB* can lead to increased body weight and contribute to obesity. This discovery will reveal new molecular pathways in weight regulation that should lead to innovative therapies, preventive measures, and insights into the pharmacogenetics of such intervention strategies.

RESEARCH DESIGN AND METHODS

Subjects' DNA, isolated from whole blood, was available from two independent cohorts of Dutch subjects: the Breda and the RIVM cohorts (Table 1). The Breda cohort comprised 492 individuals (267 women and 224 men). The level of obesity in each individual was given by the BMI, defined as weight in kilograms divided by the square of height in meters. All the patients were diagnosed according to World Health Organization criteria. There was also a cohort of control subjects available ($n = 356$) comprised of general random subjects. The RIVM cohort consisted of participants in a health monitoring project from two Dutch towns (Doetinchem and Maastricht). Subjects were diagnosed with diabetes according to venous plasma glucose concentrations (≥11.1 mmol/l for nonfasting and ≥7.0 mmol/l for fasting or were being treated with tablets or insulin at the time of the health monitoring project [1993–1997] or of a follow-up questionnaire [1998]). Control subjects were normoglycemic (venous plasma glucose <5.5 mmol/l for nonfasting and <6.1 mmol/l for fasting participants). We excluded subjects if either of their parents was not born in a European country. We calculated the BMI as weight (minus 1 kg for measured weight to correct for clothing) divided by the square of height in meters (kg/m^2).

The Breda study was approved by the University Medical Center Utrecht. The RIVM study was approved by the TNO Leiden medical ethical committee. Written informed consent was obtained from all subjects. Experiments were conducted according to the principles expressed in the Declaration of Helsinki.

SNP selection and genotyping. Using linkage disequilibrium reports from the Ensembl database (available at http://www.ensembl.org/), five SNPs (online appendix Table 1 and Fig. 1) that "tag" the common haplotypes of a region were selected (Tagger available at http://www.broad.mit.edu/mpg/tagger/) in addition to eight SNPs spanning regions between the blocks in equally spaced intervals (online appendix Table 1). Among these variants, one SNP was positioned in the 5′ upstream flanking region (SNP rs2280729) and four SNPs were positioned in the 3′ downstream flanking region (SNP rs1569128, rs1528133, rs6578931, and rs10769876). All SNPs were located in introns except for two SNPs (rs2272383 and rs3750955), which were located in the 3′ untranslated region of the gene.

Genotype analysis. SNPs were genotyped using Taqman Assay-on-Demand (Applied Biosystems, Nieuwerkerk a/day IJssel, the Netherlands). Assays were performed according to the manufacturer's specifications. The genotypes were analyzed using a TaqMan 7900HT (Applied Biosystems).

Statistical analysis. All data were analyzed using a Gaussian linear regression, including age and sex as explanatory variables in the models. The

TABLE 2
The effect of the different alleles of each of the SNPs on BMI (kg/m^2)

		Breda cohort			RIVM cohort			Combined analysis		
NCBI SNP reference	Allele	*n* (492)	Effect on BMI* (95% CI)†	*P* value‡	*n* (750)	Effect on BMI* (95% CI)†	*P* value‡	*n* (1,242)	Effect on BMI* (95% CI)†	*P* value‡
rs2280729	Minor (T)	479§	0.001 (−0.85 to 0.86)							
	Major (C)		0.30 (−0.56 to 1.17)							
rs2280726	Minor (G)	470§	−0.03 (−0.75 to 0.69)							
	Major (A)		0.88 (−0.81 to 2.57)							
rs1406095	Minor (A)	472§	0.16 (−0.64 to 0.95)		718§	−0.03 (−0.76 to 0.69)		1,190§	0.02 (−0.52 to 0.56)	
	Major (G)		0.61 (−0.26 to 1.49)			−0.36 (−1.14 to 0.42)			0.06 (−0.53 to 0.65)	
rs11041734	Minor (G)	479§	0.04 (−0.67 to 0.74)							
	Major (C)		−0.03 (−1.28 to 1.23)							
rs 4385931	Minor (C)	470§	0.06 (−1.20 to 1.32)							
	Major (G)		−0.42 (−1.54 to 0.71)							
rs2272382	Minor (A)	467§	0.08 (−0.64 to 0.80)		726§	0.41 (−0.24 to 1.05)		1,193§	0.35 (−0.14 to 0.83)	
	Major (G)		−0.97 (−2.13 to 0.20)			−1.28 (−2.32 to −0.24)	0.016		−1.12 (−1.91 to −0.34)	0.005¶
rs2242501	Minor (G)	460§	0.28 (−0.43 to 0.99)							
	Major (A)		0.17 (−1.11 to 1.46)							
rs2272383	Minor (G)	479§	0.41 (−0.30 to 1.13)		725§	0.01 (−0.64 to 0.67)		1,204§	0.21 (−0.28 to 0.70)	
	Major (A)		−0.69 (−1.69 to 0.31)			−1.26 (−2.22 to −0.30)	0.010¶		−1.11 (−1.82 to −0.41)	0.002¶
rs3750955	Minor (T)	474§	0.07 (−0.65 to 0.79)		732§	−0.20 (−0.85 to 0.44)		1,206§	−0.07 (−0.56 to 0.42)	
	Major (C)		−0.38 (−1.70 to 0.93)			+1.32 (−0.10 to +2.74)			−0.93 (−1.91 to 0.05)	
rs1569128	Minor (T)	473§	0.33 (−0.39 to 1.06)							
	Major (A)		−0.58 (−1.63 to 0.48)							
rs1528133	Minor (C)	482§	1.54 (0.45–2.62)	0.006	719§	−0.12 (−1.07 to 0.82)		1,201§	0.54 (−0.19 to 1.26)	
	Major (A)		−0.32 (−5.80 to 5.15)			−0.01 (−5.37 to 5.36)			0.12 (−3.62 to 3.86)	
rs6578931	Minor (T)	479§	0.23 (−0.49 to 0.95)							
	Major (G)		−0.28 (−1.32 to 0.76)							
rs10769876	Minor (T)	470§	0.08 (−0.64 to 0.81)							
	Major (C)		0.05 (−1.15 to 1.25)							

*All data were analyzed using a Gaussian linear regression, including age and sex as explanatory variables in the models. †The CIs were calculated using Woolf's method with Haldane's correction. ‡The *P* values were computed with a 95% two-sided χ^2 test and are not corrected for multiple testing. Corrected *P* values are 0.004 (0.05/13) and 0.01 (0.05/5) for the Breda and RIVM/combined cohorts, respectively. §Number of subjects that were successfully genotyped. ¶*P* values remain significant after stringent Bonferroni correction.

allele(s) or genotypes were then added to the model. The inference criterion used for comparing the models is their ability to predict the observed data, i.e., models are compared directly through their minimized minus log likelihood. When the numbers of parameters in models differ, they are penalized by adding the number of estimated parameters, a form of the Akaike information criterion (11). For models where the allele(s) or genotypes were found to be significant, a *P* value was computed and is presented. Differences in allele and genotype distribution in case and control subjects were tested for significance using a 95% two-sided χ^2 test. Odds ratios and the CIs were calculated using Woolf's method with Haldane's correction (12). For Hardy-Weinberg equilibrium, we compared the expected and observed genotypes in 2 × 3 tables (online appendix Tables 3, 4, and 5).

TABLE 3
Association of *TUB* alleles with obesity in the combined cohort

SNP (NCBI Reference)	Alleles	Group	Major allele [*n* (%)]	Minor allele [*n* (%)]	*P* value (allele)*	Odds ratio (95% CI)†
		BMI >30	357 (52.8)	319 (47.2)		
rs1406095	G,A	BMI <25	294 (52.9)	262 (47.1)	0.981	1.00 (0.80–1.26)
		BMI >30	415 (62.9)	247 (37.3)		
rs2272382	G,A	BMI <25	390 (68.9)	176 (31.1)	0.022	1.32 (1.04–1.67)
		BMI >30	403 (60.0)	269 (40.0)		
rs2272383	A,G	BMI <25	367 (64.8)	199 (35.2)	0.078	1.23 (0.98–1.55)
		BMI >30	507 (74.6)	173 (25.4)		
rs3750955	C,T	BMI <25	438 (77.1)	130 (22.9)	0.295	1.15 (0.89–1.49)
		BMI >30	632 (92.4)	52 (7.6)		
rs1528133	A,C	BMI <25	524 (93.9)	34 (6.1)	0.297	1.26 (0.81–1.96)

*The *P* values were computed with a 95% two-sided χ^2 test. †Odds ratios and the 95% CIs were calculated using Woolf's method with Haldane's correction.

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